PERFORMANCE, GUT MICROBIAL POPULATION AND HISTOLOGY OF BROILER CHICKENS FED PROBIOTICS AND ORGANIC ACID SUPPLEMENTED DIETS

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ABSTRACT
This study evaluat ED the effects of sodium acetate and probiotics on performance, gut histology and intestinal microbiota of broiler chickens. Two hundred and forty, one-day old Arbor Acre broiler chicks were randomly allotted to one of the four treatments: Negative control(basal), positive control (antibiotics), sodium acetate and probiotics with six replicates of ten birds each in a study that lasted for 42 days. Feed intake was not affected at starter phase. The body weight gain (BWG) of birds fed sodium acetate (648.63g/bird) was higher ($P<0.05$) compared to 463.58g/bird (negative control), 460.32g/bird (positive control), and 461.81g/bird (probiotics). At finisher phase, the feed conversion ratio of birds were not affected. The body weight gain of birds fed the different feed additives showed significant ($P<0.05$) differences. It was observed that birds fed sodium acetate had higher ($P<0.05$) BWG (965.83g/bird) compared to control (721.43g/bird), antibiotics (762.44g/bird) and probiotics (770.77g/bird) supplemented diets. Sample sectioning of the ileum of birds fed the control diet showed no observable lesion, poor section and a thinned villi. However, birds that fed with diet containing probiotics showed moderate villi atrophy, while birds fed sodium acetate diet showed no observation lesion. Lactobacillus count in birds fed sodium acetate (13.15) and probiotics (15.27) were higher ($P<0.05$) compared to other treatments. However *E. coli,* Salmonella and fungi population did not differ ($P>0.05$) significantly in birds fed different dietary treatments. The total bacteria count (TBC) observed in birds fed the negative control (basal) was higher ($P<0.05$) than TBC of birds in other diets. A healthy gastrointestinal tract of broiler chickens was achieved using sodium acetate and probiotics.
Keywords: Broiler, sodium acetate, intestinal microbiota, performance, probiotics.

INTRODUCTION
The increased pressure on livestock industry to phase out the use of prophylactic dosages of antibacterial growth promoters (AGP) in the European Union due to microbial resistance in animals and human and the prospective to do same in other parts of world has stimulated increased interest in alternative natural growth promoters (Ján, *et al.,* 2012). Such non therapeutic alternative was the use of organic acids and probiotics as feed additives in the animal production (Sheikh *et al.,* 2010). The benefits of including probiotics to poultry feed are that they stimulate immune system (Sanders, 1984), improve utilization of proteins, intestinal tract health, feed conversion ratio, strengthen beneficial microbial populations and suppress harmful bacterial growth in the digestive system, counteract adverse effect of antibiotic treatment by sustaining the population of beneficial bacteria, and also in nutrient synthesis. Organic acids and their salts are generally considered as safe and have been approved by most member states of European Union to be used as the feed additives in animal production. Organic acids work in animals, not only as a growth promoter but also as a significant tool of controlling all intrinsic bacteria, both pathogenic and non-pathogenic (Naidu, 2000 and Wolfenden *et al.*, 2007). Sodium acetate is currently approved as a flavoring agent in meat and poultry products at a level up to 25% by weight of the total formulation (FDA, 2000) and has proven useful for controlling pathogens in a variety of meat and poultry products. There is need, therefore, to investigate the effect of the organic acid sodium acetate on growth performance, gut histology and intestinal microbiota of broiler chickens.

MATERIALS AND METHODS
The experiment was carried out at the poultry unit of the Teaching and Research farm, University of Ibadan, Ibadan. Each floor pen contained one feeder, one drinker and one

brooding light. Two hundred and forty one-day old Arbor Acre broiler chicks with initial average body weight of 42 grams, sourced from a local hatchery were raised from day old to 42 days. The birds were randomly assigned to 4 dietary treatments of Basal diet (negative control), Basal diet + 0.01% Antibiotics (Oxytetracycline) (positive control), Negative control + 0.2% Sodium acetate and Negative control + 0.20% probiotics (*Lactobacillus spyrogenes* and *Saccharomyces cerivisiae*) with 6 replicate groups of 10 birds each in a completely randomized design. The feed for both starter (23.2% crude protein and 3009Kcal/kg metabolisable energy) and finisher (21.0% crude protein and 3014Kcal/kg metabolisable energy) phases were formulated based on the nutritional requirements recommended by the NRC (1994), using basically corn- soyabean meal. Ration and water were provided *ad libitum*.

The body weight gain, feed intake and feed conversion ratio were measured using standard procedure.

At the day 42, one bird per replicate was sacrificed. The contents of the lower ileum were expressed into syringes for intestinal microbial population (*Lactobacillus, E. coli, Salmonella* and fungi). A section of the ileum was removed for gut histology. All data collected were subjected to one-way analysis of variance using ANOVA programs of the Statistical Analytical System (SAS, 2008). The treatment means were compared using Duncan Multiple Range Test (DMRT) procedure of the same software.

RESULTS AND DISCUSSION

The performance of broiler chickens fed sodium acetate and probiotics is presented in Table 1.

The feed intake of birds was not significantly ($P>0.05$) different affected. However, the body weight gain (BWG) and feed conversion ratio, (FCR) were significantly different. Birds that were fed sodium acetate had significantly ($P<0.05$) higher BWG compared to those on control, antibiotics and probiotics supplemented diets. Birds fed control diet had significantly ($P<0.05$) higher FCR compared to birds sodium acetate but statistically similar to those on antibiotics and probiotics. This result is in agreement with the findings of Agboola *et al.* (2015) supplementation of broiler diets with organic acid and probiotics could improve their growth and gut morphology better than antibiotics would. This result disagrees with those of Lee *et al.* (1993) and Panda *et al.* (2000) who reported that the supplementation of an organic acid, a probiotic or any antibiotic growth promoter did not have any effect on weight gain and feed conversion ratio.

It was observed that the small intestine of birds fed antibiotics showed no observable lesion (Plate 1) and thinned villi (Plate 2). Birds fed control diet (Plates 3 and 4) indicated villi atrophy, hyperplastic crypts, a few cellular infiltrates in the propia and cryptal glands. Broilers fed Sodium acetate showed no observable lesion (Plate 5) while observations made from birds fed probiotics showed moderate villi atrophy and cryptal hyperplasia (Plate 6). The intestinal lesions characterized by severe villi atrophy changes in the surface epithelium and villi may account for some underlying infections. In the epithelium, enterocytes are responsible for the absorption of nutrients, ions, vitamins and water and are also involved in the induction of immunological tolerance to ingested peptides. Because of degeneration or loss of enterocytes from this cell lining, nutrient digestion by enzymes as well as absorption of electrolytes and nutrients is interrupted resulting in maldigestion, malabsorption and osmotic retention of water (Baker *et al.*, 1995). The similarity in the histopathological examination of the ileum of broilers fed antibiotics and sodium acetate supplemented diets is a confirmation that sodium acetate can be used in place of antibiotics. The effect of sodium acetate and probiotics on the intestinal microbiota of broiler chickens is presented in Table 2. It was observed that the lactobacillus counts in the ileum of birds fed the feed additives, sodium acetate and probiotics, were similar and significantly higher ($P<0.05$) than what was recorded for birds on other dietary treatments. The total bacteria count (TBC) observed in birds fed control diet was higher ($P<0.05$) than TBC of birds fed sodium acetate and probiotic supplemented diets but all statistically similar to those fed antibiotics. Contrary to the observation of Priyankarage *et al.* (2003), who examined the effect of probiotics on intestinal microflora and Salmonella occurrence and reported a negative correlation

between level of probiotics and Salmonella population as Lactobacillus, Streptococcus and total bacterial counts from the small intestinal wall or in the mid small intestinal contents were not altered by the addition of probiotics, this study showed that probiotic and organic acid inclusion significantly reduced the population of the total bacteria. This result shows that sodium acetate and probiotics favour the proliferation of beneficial microorganism and is similar to the findings of Thirumeignanam *et al.* (2006) which showed a decrease in the total bacteria load with concomitant increase in lactobacilli load as a result of dietary acidification. However *E. coli,* Salmonella and fungi population did not differ ($P>0.05$) significantly in birds fed different dietary treatments. This disagrees with report of Moharrery and Mahzonieh (2005) who also described that malic acid has a potential for reduction of *E. coli* populations in the intestine of broiler chickens.

CONCLUSION

The inclusion of any of the feed additives did not significantly affect feed intake, but there is need for proper intestinal microflora establishment in broiler chickens for improved absorption of nutrients as indicated in significant body weight gain in birds fed diets containing sodium acetate. The use of sodium acetate in broiler diet positively enhanced performance, intestinal microbiota and a better histological integrity of the gut compared to broilers on other dietary treatments.

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Table 1 Effects of sodium acetate and probiotics on performance of broiler chickens

Treatments	Feed intake (g/bird)	BWG (g/bird)	FCR
Control	3270.9	1185.01[b]	2.76[a]
Antibiotics	3052.2	1222.76[b]	2.52[ab]
Sodium acetate	3585.96	1613.46[a]	2.21[b]
Probiotics	3122.4	1232.58[b]	2.52[ab]
SEM	249.3	50.29	0.16
P Value	0.46	<0.0001	0.15

ab- Means for treatments along a column with no common superscript differ (P<0.05) significantly F.I- Feed intake, BWG- Body weight gain, FCR- Feed conversion ratio, P value- Probability value, SEM- Standard error mean

Table 2 Effects of sodium acetate and probiotics on intestinal microbiota of broiler chickens.

Treatments	Lactobacillus (x 10^4) CFU	*E coli (x 10^4) CFU*	Salmonella (x 10^4) CFU	Fungi (x 10^4) CFU	TBC (x 10^4) CFU
Control	6.63[b]	5.11	6.15	2.39	28.42[a]
Antibiotics	6.13[b]	5.86	4.44	1.99	19.89[ab]
Sodium acetate	13.15[a]	4.2	4.83	2.23	11.83[b]
Probiotics	15.27[a]	5.52	6.34	1.89	12.32[b]
SEM	1.12	0.68	0.94	0.45	3.91
P Value	<0.0001	0.37	0.4	0.85	0.02

[ab]Means for treatments along a column with no common superscript differ (P<0.05) significantly. CFU- Colony forming unit, TBC- Total bacteria count, P Value- probability value, SEM-Standard error of mean.

Plate 1: No observable lesion crypts

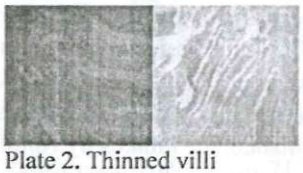

Plate 2. Thinned villi

Plate 3. Villi atrophy and hyperplastic

Plate 4. Villi atrophy and a few cellular infiltrates in propia and cryptal glands

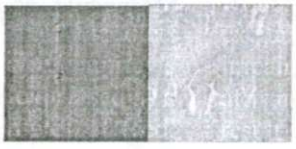

Plate 5: No observable lesion – Sodium acetate

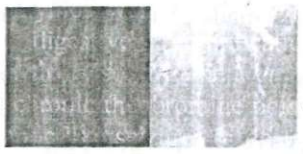

Plate 6: Moderate villi atrophy and cryptal hyperplasia - Probiotics